COMMENTS FROM REMEDIUM / PARAMETRIX

Draft Libby OU3 Phase III SAP Review
Comment/Revision Summary

Section #	Page, #	Comment / Suggested Revision
Figure 4-1 (CSM)		Multiple comments: 1. Surface soil, duff box (uptake into tissues): adult life stages of amphibians may also be exposed and are not shown. 2. On the CSM, what does "direct contact" mean as an exposure route? Is this dermal exposure? For plants, assume it means roots in contact with soil or particulates on the leaves? 3. Why are amphibians assumed to be exposed only through ingestion and not via inhalation or "direct contact?" This makes little sense given agency concerns expressed that contact of developing amphibian limb buds with LA could be of concern to metamorphosis, and that a 96-hr FETAX test that examines exposure from multiple pathways including direct contact should be conducted... 4. Surface water fate as shown is incomplete. Specifically, we would expect some settling of LA out of surface water bodies (given specific gravity) into sediments. Some resuspension will occur depending on the type of water body (lentic versus lotic). The settling (and potential for resuspension) is important from an exposure perspective and is not reflected on the CSM. 5. Wetland plants are not shown in the CSM though wetlands are reported to occur in and around the tailings pond and along Rainy and Fleetwood creeks. 6. Reptiles are not indicated as a receptor on the CSM though 7 species are reported to occur in the Libby OU3 area according to Problem Formulation. Their exposure will likely be less than that of fish (or amphibians which have a water exposure phase and a terrestrial phase) but it is unclear why they are omitted from the CSM.
4.1.1	27	Aquatic receptors listed are fish and invertebrates. Amphibians are missing. Amphibians typically have aquatic life stages (larval) with terrestrial adult life stages (varies by amphibian Order, e.g., Anura, Urodela, Gynmophiona). Additionally, reptiles are not listed as terrestrial receptors and should be. Some reptiles also have aquatic exposures if present (i.e., turtles).
4.1.2.1	27	The primary assessment endpoints appear to be some "protection" of population or community, with the surrogate endpoints of growth, reproduction, and survival. First, it is not a given that all ecological risk assessments have this same assessment endpoint. However, if this is the primary endpoint for this assessment, it would be helpful if there were a more explicit statement about what population/community attribute is to be protected, such as maintenance of population densities (or growth rates?) at levels not significantly different from reference populations. One would then presuppose that if there is no effect of LA on growth, survival or reproduction of individuals, then there would be no population level effect. However, the converse is not true. There can be some degree of reduction in these three attributes without changing the population growth rate or density, although the age/sex ratios may shift as a result. Carcinogenicity is an effect endpoint rather than an assessment endpoint (it is not a valued characteristic to be protected). This text should be deleted.

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4.1.2.2	28	Bullets: hazard quotients are not measurement endpoints and text should be corrected. Measurement endpoints are of several types including measures of effect, exposure and of receptor characteristics. When defining Hazard Quotients, it would be helpful to be specific about the TRVs, that is, that they are thresholds of effects on growth, reproduction, and survival (which are your assessment endpoints rather than the HQs).
4.1.2.3	28	Lines of Evidence – will all lines of evidence be given the same weight? What are examples of the kinds of lines of evidence that will be considered? Are some inherently stronger/weaker than others, or does this depend upon the quality/quantity of data gathered in support of that particular Line of Evidence (or both?).
4.2.2	29	This section is focused on LA in surface water, but what about other chemicals (metals)? Not sure if other chemistry has been collected in previous first flush samples collected, but if so, then these samples would also need to be consistent from an analytical perspective. Importantly, chemicals other than LA present in surface water may result in potential risks to aquatic life (and affect populations). Accordingly, it is not appropriate for a sole focus on LA as a causal factor in the investigation of risk potential to aquatic life. This is a global comment on the DQOs which seem to be wholly focused on LA concentrations only as a causal factor. Section 3 of the SAP (Human Health) includes a table with a screening level risk assessment of surface water for other mine waste. Why isn't this done for ecological receptors to provide assurance that it is appropriate to focus only on LA concentrations (i.e., that metals and other mine waste can be "ignored") in the DQOs? Aquatic life is particularly sensitive to some metal constituents.
4.2.2.2	30	The seven step DQO process outlined in this section (and other DQO sections in the SAP) is too brief, does not match the steps identified in the 2006 guidance, nor do the Phase III SAP DQOs present the depth of information required in that guidance (USEPA 2006; EPA/240/B-06/001). Some DQOs presented are much more detailed than others; for example the mammal DQO. This level of detail is preferable in all of the DQOs (fish, amphibians). We believe all the DQOs need to be consistent in the types of information presented (see EPA's 2006 DQO guidance). For example, risk questions are generally not presented nor are any alternative outcomes related to risk questions presented. Decision criteria should be spelled out clearly to ensure data collected are used for their intended purpose (i.e., to answer the risk questions identified). Step 1: would prefer the statement "to protect fish from unacceptable risks" rather than "to protect fish from ecologically significant adverse effects." There are too many unqualified terms in the way it currently is written. For example, what do we mean by "ecologically significant?" Does this infer some statistical significance or something merely biological? And how is the level of significant determined and by whom? By using the term "unacceptable risks" we are explicitly acknowledging that there is a policy determination of what will be considered "unacceptable." Step 3 and other steps of this DQO reference a section (4.1.2.4) that does not exist. This was probably meant to be a reference to section 4.2.4.2.

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4.2.2.2	30	General Comment: This DQO is focused on LA concentrations in surface water. However, metals or other chemical constituents may also be contributory to risk. Other chemical constituents have been collected in surface water in previous years. Why aren't other chemical constituents and their potential to affect risk woven into this DQO? Each DQO should be stand alone and not cross referenced to another DQO as is done in steps 5-7.
4.2.2.2	30	Singular focus on LA concentrations rather than LA and other mine waste throughout this SAP is problematic from a risk perspective. Metals and other mine waste concentrations should be specifically included to ensure that aquatic risk findings (in particular) are not attributed to other types of exposure.
4.2.2.3	30	Please identify if there is a stream gauge(s) that is being monitored and note its location and criteria for establishing peak flows. Where is a map showing the sampling locations?
4.2.4.1	34	"Density" is defined as numbers per unit area. The information provided in this summary is of the number of fish captured per stream that is then translated into a population density using the referenced algorithm. However, there is no mention made about the "unit of area" that is used in the density calculation. Are all the streams the same width? What is the reach (length) of the stream that is represented by this density estimate? This information would be critical in understanding what these data mean. Additionally, while it is useful to look at this for all fish species together, additional information would be gained through conducting the same exercise for each species. It may be that one species is less affected than others (or vice versa).
4.2.4.1	35	Why would one set of fish densities (>65 mm) be corrected for electroshocking recovery while another set of fish densities (<65 mm) is not? Text does not explain and should. What impact does this have on the draft findings for fish density that are presented?
4.2.4.1	Fig 4-3	Which ones are the reference stations? At which station were the brook trout captured?
4.2.4.2	35	DQO Step 2 – EPA guidance asks that the various potential outcomes be identified, along with what the Agency response is likely to be for each outcome. DQO Step 3 – it would be helpful if the word "demographics" were defined. Generally, this refers to density (#/unit area), age structure, sex ratios, stage/age-specific survival rates and stage-specific reproductive rates. Also, language here on "presumptive causative agent (LA)". Why aren't other mine waste constituents included here? See comment above on the need for this SAP to consistently address LA in the context of other mine waste concentrations to ensure that all potentially adverse exposures are properly understood.
4.2.4.2	36	DQO Step 4 – the information provided specifies which creeks/streams will be sampled. But what is the spatial extent of the decision that will be made? Will "unacceptable risk" be attributed to the entire OU if effects are found at one stream? Or all streams? Or will risk decisions be made separately for each individual stream? What about streams that aren't sampled? Will they be included in any risk decisions by inference? How?

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4.2.4.2	37	DQO Step 6: Exactly what data is going to be evaluated here? Specifics are lacking. Text recognizes that statistical tests will be of limited use given small sample size (two years of sampling only). Additionally, the statistical power of any statistics is also going to be low with only two sampling seasons. Is this setting up the argument for a multi-year fish population study? This part of the DQO should discuss what the likely outcomes are to establishing risk potential based on two years of this limited population data. Ideally this would be in a decision matrix showing possible outcomes on population and the corresponding decision that would be made.
4.3	39	See previous comments on need for all DQOs to evaluate LA with other mine wastes to ensure outcomes can be properly understood from a total exposure perspective. This DQO makes an a priori assumption that only LA concentrations are the focus of the data collections to support the ecological risk assessment. The reviewer is given no context to understand if this assumption is correct.
4.3.1	39	How many years of macroinvertebrate community data are required? Why isn't this specified?
4.3.2	39	Where is a figure showing Phase I, Phase II and Phase III sampling locations?
Table 4-3		What about the concentrations of other mine waste constituents in sediment? Again, aquatic life, including benthic invertebrates, can be particularly sensitive to some metals. Why the singular focus on LA concentrations?
4.3.4.1	41	Where is a figure showing the 2008 and 2009 sampling locations? Should be added. Additionally, benthic invertebrate serber data that were collected are also being evaluated and compared to Montana's findings for the montane region. Additionally, serber data are also being compared to selected data (i.e., closest to Rainy Creek) collected in the Kootenai by Vinson.
4.3.4.2	42	Focus again is on LA concentrations and other mine waste constituents (particularly metals) are ignored. DQO Step 3 – because this is a community assessment and not a population study, it is not appropriate to say that benthic invertebrate "demographics" will be collected. Rather, the statement would be that benthic macroinvertebrate community metrics will be collected. Similarly "community demographics" should be "community metrics." DQO Step 4 – see above comment about the difference between the spatial bounds of the data collection and the spatial bounds of the inferences. Both should be discussed in this step to demonstrate that the collection sites are sufficient for the spatial extent of the inferences to be made. DQO Step 5 – A weighting scheme has not been presented and should be if a "weight" of evidence is to be used. This is actually a global comment on the Phase III SAP.
4.3.4.2	44	First line is a typo and refers to fish rather than macroinvertebrates.

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4.4.1	45	Problem formulation does not discuss a weight of evidence approach as text here notes. Problem formulation discusses "lines of evidence". Will a weighting scheme be used as text indicates and where are the weighting factors defined for each line of evidence?
4.4.2	45	Assessment of existing data - While there are no data available on the effects of LA on wild rodents (from this site or elsewhere), it would be very helpful to provide a summary of potential histological and/or gross pathology caused by asbestos (in general) to rodents. This would provide a basis from which inferences could be made about the potential for similar effects to occur in small mammals on site. For example, a discussion of differences in lung structure between humans and other mammals that may influence depositional areas for LA fibers would be instructive for conducting the histopath exam (i.e., human lungs are symmetric, while rodent lungs are asymmetric in regard to bronchiole branching patterns). Deposition and distribution of fiber length also may differ between humans and small mammals. Thus, the relative potential for pleural effects versus interstitial effects in the lungs may differ significantly. Some discussion about potential cancer types (if any) to be aware of would also be helpful (i.e., are mesotheliomas a possibility? Why/why not?). Other effects such as inflammation and fibrosis should be discussed. Finally, what is the evidence (if any) of effects of asbestos on organs other than the pulmonary system (i.e., GI cancers)? This type of general review/summary would provide the background information for what type of risks to small mammals may occur from LA exposure and, therefore, provide support for the hypotheses to be examined by the proposed study design. See Page 53 for some of this review.
4.4.2	45	DQO Step 1 – while it is true that "the risks to small mammals are not known," it also is true that there is some reason to believe that LA exposure may be harmful to these animals (otherwise, there would not be a recommendation for these studies). Hence, the review of potential effects suggested above, which then could become part of the Problem Statement ("It is hypothesized, based on information from humans and laboratory rodents, that LA can cause adverse effects to small mammals given sufficient exposure magnitude and duration").
4.4.3	45	DQO Step 2 - the final statement about "local population growth, reproduction, and survival" is not clear. One can have a measure of population growth (defined as "r"; the incremental change per year in number of individuals in the population) which is a function of reproduction rates and survival rates. This needs to be stated more clearly. Furthermore, the type of data that will be collected (see DQO Step 3) will not address either population growth or reproductive/survival rates. Therefore, either the question being addressed OR the data being collected need to change so they are compatible.

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4.4.3	46	DQO Step 3 – Given the amount of data that exists for duff samples, only a single duff sample will be needed at the mine site trapping area (a composite) and at the reference site trapping area (also a composite). DQO Step 4 - Spatial bounds: Only a single area downwind of the mined area should be sampled because the mined area has little if any habitat to support small mammals. DQO Step 4 - Temporal bounds. Sampling should occur in late summer when populations are at peak levels. Gender and reproductive state can be determined and recorded during necropsy. Text should be revised. DQO Step 4 – Target Species. *Peromyscus* and *Clethrionomys* should be the target species and these should be clearly stated here for all the reasons currently discussed.
4.4.3	48	DQO Step 5 - The table presented on this page provides good detail that is absent from other DQOs.
4.4.3	49	DQO Step 7 - Possible typo: Text refers to Figure 4-9 and discusses panel 1 as low variability. However, text notes Panel A has a CV of 0.1 while Figure 4-9 has a CV shown of 0.2. Should the text refer to Panel A as a CV of 0.2? Text in this DQO step indicates that 20 animals per species should be collected. Further, because the assumption *a priori* is that the reference site will have few if any asbestos lesions, the statistical power of the comparisons should be higher with a smaller number of animals collected. Only 10 animals per species should be needed. Figure 4-9 should be revised accordingly.
4.4.4	50	What are the decision criteria for determining if the duff samples are "elevated"? Too many duff samples are proposed for collection. Only a single composite sample is needed downwind of the mined area where trapping in OU3 will occur, and a composite at the reference area. The DQO decision criteria here should reflect that the concentration of the composite sample downwind of the mined area will be within the concentration range of the three highest existing duff sample stations (15-02, 45-02, 45-03). This needs to be defined more specifically here. Further, the timeline for "rapid" turnaround of this sample must be clearly specified. People will be in the field collecting the sample and there should be no expectation that they will be "waiting" in the field for this result.
4.4.4	52	Trapping effort. Because only 10 animals per species will be needed this text should be revised accordingly.
4.4.4	52	Measurements on Mammals Collected in Traps. This text should be removed completely. All measurements and gender determinations will be done by staff on euthanized animals only. Measurements on Mammals Collected and Sacrificed. Weights will be taken of euthanized animals only. In the lab, at necropsy, sex will be determined. Females will have the uterus removed before weighing. A pesola type scale will be used for weights. Photographs will also be taken of each animal. Eye lens will be removed from each euthanized animal and frozen for later aging if this becomes necessary (it may not be). Photographs will be taken of each euthanized animal. Text should be added to reflect these changes.

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4.4.4.	53	Preference will be given to pregnant and lactating females. This text should be clarified. Are we only interested in keeping pregnant females? What about females that have already had a litter but are not currently pregnant (necropsy should tell us this)? These animals should be retained as well...
4.4.4	54	Disagree with the identified euthanasia method for small mammals. CO2 asphyxiation in the off-site necropsy lab is recommended because (1) pulmonary pathology is not associated with CO2 asphyxiation based on discussions with a qualified histopathologist, (2) it is more humane to the animals, and (3) it is easier for necropsy staff. This method is recommended over cervical dislocation by the American Veterinary Medical Association. Text should be revised accordingly.
4.4.4	54	Only a single composite duff sample will be collected downwind of the mined area and a single composite duff sample at the reference site. Text should be revised to clarify.
4.5	56	Data on birds will not need to be collected at OU3 because the small mammals represent a worst case exposure and because avian physiology will render them less sensitive to the effects of asbestos than mammals. Please refer to Attachment 1 for a full explanation of the comparative physiology between birds and mammals that will serve to reduce avian toxicological sensitivity to asbestos.
4.6	65	General Comment: Field data on early life stage amphibians (eggs, tadpoles, metamorphs) should not need to be collected because amphibians will be less exposed relative to early life stage fish, which are already being evaluated *in situ* and using laboratory toxicity bioassays. For example, early life stage fish and amphibians will both have ingestion exposures *in situ*, however, respiration exposure to LA will be reduced for amphibians because they respire through a combination of primitive gills, air gulping through lungs which reduces their LA exposure, and through gas exchange through the skin (also reduces LA exposure). Though the expressed concern in collecting the field data is for establishing the potential effects of LA on metamorphosis and malformations given limitations of the FETAX bioassay (doesn't go through metamorphosis), the data proposed for collection (and for comparison with reference amphibian data) cannot be used with any defensibility to establish such causality without the conduct of additional (and significant) research given the myriad of natural factors (pathogens, UVb radiation, parasites, etc.) that are known to cause these effects naturally. Further, amphibian populations are well known to be highly variable from year to year and the likelihood of inaccurately attributing (statistically) causality to LA rather than other factors cannot be discounted. Accordingly, in our judgment amphibians need only be evaluated if results of the updated rainbow trout bioassay indicate a cause for concern given our judgment that the exposures of early life stage fish will be greater than the exposure of early life stage amphibians. A 96-hour FETAX bioassay is also proposed. This test could be run but should use Rana species, which occurs at the Libby Site, rather than Xenopus, which does not. Any testing should be postponed until the methods and exposure challenges from the rainbow trout pilot study are concluded to inform this test.

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4.6.6.2	65	DQO Step 2 - Here again the effects from other mine waste are ignored, as is a practical consideration: there are a host of other factors, well documented (Linder et al 2003) that can affect amphibian populations in the field, including UVb and fungal infections to name but a couple. How can it be accurately ascertained whether any observed outcomes for amphibians based on field data have linkages to LA versus to other environmental stressors (many of which can work in synergy)? Linder, G., S.K.Krest, D.W. Sparling. 2003. Amphibian Decline: An Integrated Analysis of Multiple Stressor Effects. SETAC North America. Pensacola, FL.
4.6.3.1	69	The USFWS data referred to include abnormalities from natural and physical factors, including malformations resulting from pathogens, parasites and UVb for example. How specifically are these data going to be used to "interpret" site observations? More detail on how these data are to be used is required. Decision criteria for using this data should also be provided.
4.6.3.2	69	DQO Step 1 - Abnormalities at reference sites are considered ecologically significant. What is meant by ecologically significant in the comparison of reference and OU3 amphibian data? This term is loosely used and should be avoided or more specifically defined. DQO Step 2 - Where are the risk questions identified and the alternative outcomes? See Step 2 in USEPA 2006 DQO guidance. This and other DQOs in this SAP appear to be lacking important details.
4.6.3.2	70	DQO Step 5 - it is not certain how a conclusion that LA is causal (as stated here) can be made when other mine waste is not also examined as well as other water quality parameters and natural factors that affect abnormalities and that could be site specific. Text says that if the evidence is considered "uncertain" it will be given "low weight" in the weight of evidence evaluation. A comment throughout this SAP is that the weighting scheme for the WOE evaluations referred to throughout is not provided. This must be provided and discussed.
4.6.3.3	71	Though we disagree (see earlier comment) that the amphibian work identified is required, we note a number of questions and inconsistencies (i.e., to other proposed studies) with the study design as presented. For example, abnormalities that may be observed at Libby may have nothing to do with LA concentrations, regardless of the USFWS data base, and may in fact be associated with other site factors. Also, why wouldn't amphibian tissues be collected for LA analysis as they are in other site studies? The real question becomes how are site factors other than LA concentration going to be considered (i.e., other mine waste; parasites, pathogens, fungal infections, other water quality parameters, etc.) in assessing causality for any observed malformations absent histology, tissue, or site water quality data? Many of the factors that contribute to abnormalities in amphibians are known to act in synergy. Why collect 50 – 100 metamorphs per species? Some indication of potential power of comparison of incidence of malformations among locations is needed to justify this number (this analysis was presented for mammals). See comment on mammal DQO above that indicates only 10 animals each at OU3 and the reference site would be needed given the assumption of low incidence of LA occurring at a reference site. Why wouldn't this also be the case for amphibians?

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6.1	89	Throughout section – include "pathology laboratory" after "analytical laboratory" as appropriate.
6.1.1	89	What type of data should be recorded relative to photographs? In the field notebooks or on separate datasheets?
6.1.2	89	Sample containers – this applies to all media collected for LA analysis, but is not applicable to necropsy tissue collection. Sample jars for tissue collection will be provided by the pathology laboratory, preferable prefilled with formalin. There is no Table 6-1 provided.
6.1.3	89	Should specify holding times for tissues for histopathology as < 1 year.
SOP: BMI		
4.1	2	The equipment list should include; a velocity meter, a meter tape, meter stick, ph meter, DO meter, turbidity meter, and a conductivity meter.
6.3.1	6	For the Surber sample collection it should be noted that 90 seconds (1 ½ minute) should be spent disturbing the substrate with each sub-sample. Therefore, a total 270 seconds (4 ½ minutes) will be the total time spent at each location.
Habitat Assess.	8	Velocity measurements should be more quantitative. The substrate size evaluation should be quantitative, an SOP could be added. Riparian cover could be measured using a densitometer.
	19 Physical Characterization/Water Quality Field Data Sheet (Pg. 2)	Velocity should be more quantitative. At least 5 measurements if not 10 measurements for each location.
	20 Physical Characterization/Water Quality Field Data Sheet (Pg. 3)	The substrate size evaluation should be quantitative, a Pebble count could be used. Assuming the goal would be to characterize the entire reach then a zig-zag approach should be used.

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SOP Mammal		
3	3/18	No state collection permit is noted in the equipment list as is done for other SOPs. Why are the equipment needs for necropsy and tissue processing not specified here?
4.2	4/18	When is a field reconnaissance proposed to occur? Why not just say here, deer mouse (*Peromyscus maniculatus*) and the southern red-backed vole (*Clethrionomys gapperi)* are the target species.
4.5	5/18	Instead of sketching the location/orientation of each trap, a photograph can be taken along with a GPS recording.
4.6	5/18	Trap nights is defined incorrectly. The number of traps multiplied by the number of nights equals trap nights (not number of traps multiplied by number of trap nights as stated). Additionally, only 10 individuals per species should be targeted based on a revised statistical power analysis (see small mammal DQO comments) so the language throughout this SOP should be revised.
4.7	5/18	Drop need for setting traps out for almost a week ahead of trapping to avoid trap happy individuals. This is not a population survey so with the emphasis on tissue collection we shouldn't care if we catch trap happy individuals (makes the job easier). Checking the traps. Language should be revised to indicate placement of traps at dusk, but then check trap first at dawn, then check traps after the first two hours of sunlight and again at dusk.
4.7	6/18	Traps should also have assigned bags for transporting the trap on and off site.
4.9	7/18	General Lifestage. This information does not seem particularly important for collection in the field (body measurements are specifically indicated for example). Handling small mammals in the field invites loss of the specimen. All measurements should be done only after the animal has been euthanized by CO2 asphyxiation in the lab. Photos and other measurement information can also be collected at this time. We do not want field personnel getting bitten or otherwise put at risk.
4.10	8/18	CO2 asphyxiation is the preferred method and will not result in any pathology to the lungs. This is the recommended approach as well by the AVMA. Revise SOP accordingly. Additionally, eye lens' will be collected, frozen and stored for evaluation only if needed. Please revise text to reflect.
4.10	9/18	There is no mention of the methods needed to transport the animals retained for necropsy to the on-site processing laboratory.
4.11	9/18	For the lungs and kidney, one whole organ should be placed into the formalin. The other can be sectioned longitudinally to look for gross lesions, and then submitted for LA analysis.

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4.11	10/18	Lungs – it may be difficult to ID what is a "lower lobe" of the lung. For example, rats have 4 lobes in their right lung and only one in the left. Unclear how many lobes a deer mouse or a vole has in their lungs, but LA fibers may get deposited asymmetrically between the 2 lungs and within each of them. SOP should either save one lung in formalin and the other submitted for LA analysis (the same one in all individuals) or split the lungs laterally into a "top" and "bottom." Neither of these approaches is perfect as it is not known where the asbestos fibers are most likely to deposit within the different lung lobes. Further discussion may be needed on this point. The scale used for the purpose of weighing tissue specimens will have to be very accurate (~0.1 mg). Therefore, the scale will have be placed in an environment where it is unaffected by air flow. Highly accurate scales are affected by airflow.
7.2.2	93	How will histology data be handled in the field in terms of uploading data? Or is this not necessary?
SOP: Amphibian		
		Per earlier comment on amphibian DQO, we do not agree that the specified field work for amphibians is necessary or that it will provide particularly meaningful data given the significant limitations in establishing causality of observed field effects to LA over other natural factors. Comments on the SOP are included pending resolution of this issue.
3	3/20	Safety equipment is not listed as in other SOPs. This should be added.
6	4/20	How many weeks is the survey to be done? Section 7.1 says the survey should be conducted "once in April" and again in "late May". These seem to be saying something different. Clarification is required.
7.1	4/20	Survey Locations. What is the perimeter length of the different ponds for surveying at OU3 (this information should be added) and can the survey locations be realistically 1/2 mile apart as indicated?
7.1	4/20	Survey Period: here text says once in each of two months. Earlier text says "weeks". The amount of time for "surveying" and "monitoring metamorphs" is too vague and requires more clarity and specificity.
7.1	4/20	Survey Conditions. Will temperatures at night be at least 42 degrees at night at OU3 in April? Snow may not even be melted yet so is this temperature requirement realistic for the time of year being specified? Nighttime temperatures for Libby in the April – May timeframe should be summarized to support this temperature requirement.
7.1	5/20	Life histories for the frogs/toads indicate only one will actually vocalize in a manner that may be consistently heard. Both auditory and visual techniques will be required to complete the survey. Why isn't this discussed? There should be an additional form for recording visual encounters; where is the form?

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8	5/20	Text says a "weekly" basis for monitoring. How many weeks? A time limit needs to be identified.
9.1	7/20	Text says 50-100 metamorphs "per site". We agreed to one site and if time permitted two. Also, what is the basis for this number of metamorphs? Can less be collected? There does not seem to be a rationale for this high number of organisms, nor the requirement for samplers to keep coming back if needed to attain the minimum (50). Text says tadpoles should be collected as well. Metamorphs are not tadpoles. Which is it?
9.2	9/20	The Gosner stage chart is not included in this SOP and should be added.
10.1	10/20	End of page: text indicates any abnormal individuals should be documented as described in Section 8.0. Section 8.0 is Monitoring For Metamorphs. Shouldn't this refer to Section 10.2?

Attachment 1: Sensitivity of Birds to Libby Amphibole

As part of the 2009 Phase III sampling program for the Libby Mine Site, bird sampling was considered to determine if birds are potentially at risk from Libby amphibole fibers. There are no studies of the effects of asbestos on birds, and only one published study on particle deposition in the avian respiratory tract. Therefore, empirical comparisons of sensitivity of birds to mammals following exposure to Libby amphibole are not possible. However, because of differences between the physiology of the avian and mammalian respiratory systems, gastrointestinal tracts, and kidneys, it is probable that birds will be less affected than small mammals by inhalation of the Libby amphibole. The comparisons are described below.

Respiratory Comparisons

Asbestos fibers are known to lodge in the lungs of mammals, with the long, thin Libby amphibole fibers depositing mainly in the lower airways and alveolar regions (ATSDR, 2001). As a foreign antigen, they attract alveolar macrophages and pulmonary neutrophils, and interact with epithelial cells and pleural mesothelial cells, setting off an inflammatory cascade response and eventually a walling-off of the fiber from the lung tissue. This results in pulmonary interstitial fibrosis and collegen deposition, with progressive lung stiffening and thickening and calcification of the pleura and, eventually, a reduced ability of the lungs to expand, thus decreasing gas exchange and oxygenation of the blood. (ATSDR, 2001). Production of reactive oxygen and/or nitrogen species may result in carcinogenesis, particularly of the pleural mesothelium.

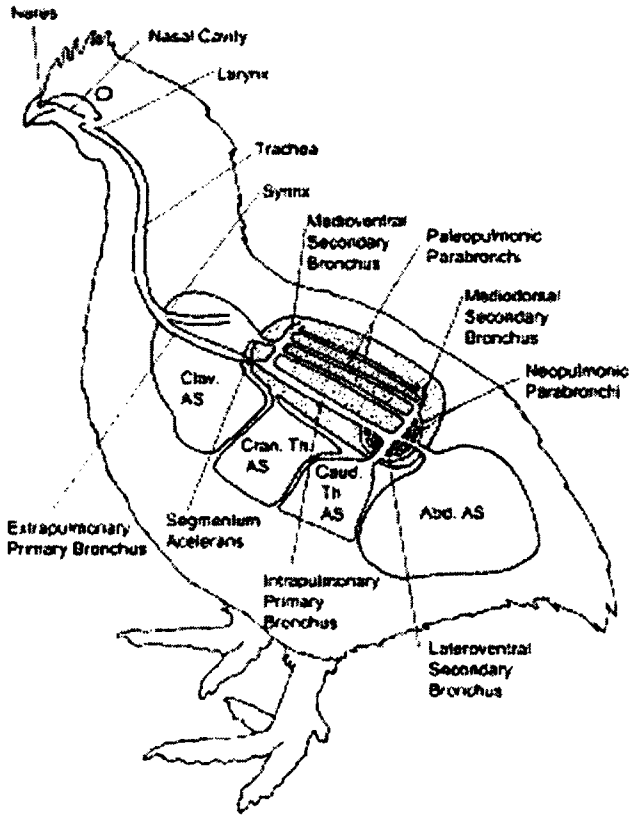

FIGURE 1. General organization of the respiratory system in the chicken. Clav. AS = clavicular air sac; cran. th. AS = cranial thoracic air sac; caud. th. AS = caudal thoracic air sac; Abd. AS = abdominal air sac.

Birds, on the other hand, have relatively small lungs that do not expand

upon inhalation (Brown et al., 1997). Instead, the air is pulled through the lungs by the bellows action of the air sacs (see Figure 1; from Fedde 1998). The air flows through the lungs in a single direction on both the inhalation and exhalation parts of the breathing cycle. There are no blind alveolar sacs, as in mammalian lungs, and the air simply passes through a series of smaller and smaller bronchi, which are highly vascularized for efficient gas exchange. Because the lungs do not expand during inhalation, pleural thickening and calcification (if any) or interstitial fibrosis that may be caused by asbestos fibers would have no effect on respiratory efficiency.

Although birds have prominent bronchus-associated lymphoid tissue, they lack surface alveolar macrophages (Reese et al., 2006). Instead, the phagocytic function of macrophages is fulfilled by epithelial cells. Particles move into liposomes within the epithelial cells or they may move through to the interstitium, where they are picked up by interstitial macrophages (Reese et al., 2006). The lack of alveolar macrophages suggests that birds may not respond as aggressively to particles that remain within the lungs, and therefore may have less interstitial fibrosis. Further, mid- to large sized particles ($\geq$ 10 μm) deposit primarily in the abdominal air sacs and caudal (rear) bronchi (Stearns et al., 1987) rather than in the lung parenchyma. Because the air sacs are made of connective tissue with very little vascularization, inflammation and fibrosis as a result of fiber deposition does not appear likely.

Birds have a high requirement for oxygen, as flight is the most metabolically expensive form of locomotion on a unit-time basis (Brown et al., 1997). However, the effective ventilation in birds under resting conditions is 30 – 160% higher than mammals of comparable size, indicating the much higher gas exchange efficiency of the avian lung (Brown et al., 1997).

Gastrointestinal and Kidney Comparisons

The avian gastrointestinal (GI) tract is similar in structure to that in mammals, so likely will experience the same type of response to asbestos ingestion. However, birds do not have an epithelial mucocilliary transport mechanism for removing particles from their trachea and upper bronchi (Fedde, 1998), and so may experience less GI exposure through pulmonary clearance than do mammals. Although the gross morphology of the avian kidney differs from that of mammals, the nephron is still the functional unit, with the same basic structure of glomeruli that filter the blood and renal tubules to reabsorb water. Thus, there is no reason to believe that the sensitivity of response to renal asbestos exposures would differ between birds and mammals.

Summary

In summary, birds are less likely than small mammals to suffer from respiratory effects of Libby amphibole because:

- Their lungs do no expand during breathing so pleural thickening or calcification is not a problem;
- The flow-through construction of their lungs would result in particle deposition occurring primarily in the air sacs;
- Air sacs are not very vascularized, so inflammation generally does not occur; and
- They do not have alveolar macrophages, so may experience a reduced intestinal inflammatory response.

Birds are not likely to differ from mammals in regard to sensitivity of gastrointestinal tract or kidney exposures.

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